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NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES

ANNUAL REPORTS

INTRAMURAL RESEARCH PROGRAMS

OCTOBER 1, 1988 TO SEPTEMBER 30, 1989

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PREFACE

The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is responsible for research on a broad range of diseases and conditions that at one time or another involve almost every family in the Nation. Most of these diseases are long-lasting. Many are severely disabling. They generate a tremendous burden of human suffering and economic loss, both to the individuals and to society, as a whole.

In its extramural research programs, the Institute supports a wide spectrum of both basic and clinical research, and research training, in the important fields of rheumatology, muscle biology, orthopaedics, bone and mineral metabolism, and dermatology.

Major avenues of arthritis research include: humoral and cellular immune abnormalities in the pathogenesis of rheumatic disease; mechanisms by which mediators of inflammation, such as the prostaglandins and leukotrienes, promote and regulate inflammatory reactions in arthritis; immunogenetic factors underlying susceptibility to arthritis; ultrastructure and pathophysiology of collagens, proteoglycans, and other components of connective tissue; and metabolic derangements underlying osteoarthritis and gout/pseudogout.

Musculoskeletal disease research includes studies of the basic structure of bone and cartilage; metabolic bone disease; fractures and the healing process; prostheses and relevant biomaterials; biomechanics and bioelectric phenomena; bone transplantation; the physiology of normal and abnormal gait; and inherited connective tissue disease.

In dermatology, investigations are under way on a wide range of diverse problems, including psoriasis, acne, vitiligo, atopic dermatitis, and fatal blistering "bullous" diseases. Employing new laboratory techniques, scientists are attempting to clarify the roles of immunopathology, phototoxicity, and metabolic abnormalities in the etiology of these cutaneous disorders.

The Intramural Research Program of the Institute consists of two major investigational entities: the Arthritis and Rheumatism Branch (ARB) and the Laboratory of Physical Biology (LPB). Dr. Henry Metzger, an internationally-renowned immunologist, is the Director of Intramural Programs of NIAMS. Dr. John H. Klippel, a highly talented clinical investigator of rheumatic diseases, is the Institute's Clinical Director.

The Arthritis and Rheumatism Branch was established over 30 years ago as a major intramural component of the National Institute of Arthritis and Metabolic Diseases. It has been one of the outstanding centers for laboratory and clinical research and research training in rheumatology, basic immunology and related areas. Research in the Branch is directed by six Senior Investigators and covers a broad spectrum of disciplines: biochemistry, molecular biology, genetics, cell biology, immunology, pathology, pharmacology, and clinical investigations. In addition to collaborations within the Branch, there are many cooperative projects with other groups at the National Institutes of Health. The Branch is organized into four sections.

In the Section on Cellular Immunology, genetic and cellular abnormalities that underly human and murine autoimmune diseases are explored. Emphasis is placed on research on systemic lupus erythematosus. Studies cover the range of fundamental investigations on the nature and development of B lymphocyte repertoires to the development and evaluation of experimental therapies.

In the Section on Connective Tissue Diseases, the etiology and pathogenesis of auto-immune diseases are explored. In particular, an excellent rat model of rheumatoid arthritis is being studied--recent emphasis being on the effect of the hypothalamic-adrenocortical axis on the course of the disease. Polymyositis in humans and mice is also being thoroughly investigated, with special emphasis on the use of highly sensitive genetic probes to look for possible viral etiologic agents.

In the Section on Chemical Immunology, basic questions dealing with the mechanism by which cells bearing antibody receptors are activated, are under investigation. The receptor for immunoglobulin E on mast cells serves as a model system and the genes for this receptor were recently cloned and successfully expressed. Structure-function relationships are currently being explored by molecular genetic approaches.

In the Orthopedic Research Unit, fundamental aspects of bone development are being explored using molecular genetic techniques for identifying growth factors, and for other inter-cellular messengers. Special emphasis is being given to the progressive changes during repair of fractures in experimental normal and diabetic animals.

The other major component of the NIAMS Intramural Program is the Laboratory of Physical Biology (LPB), the underlying scientific disciplines of which are biophysics and structural biology. The Laboratory studies biological problems using a broad range of physical techniques. They include X-ray diffraction, high resolution mechanical recording electron microscopy, image processing, surface film techniques, and radiation inactivation analysis. The Laboratory consists of three Sections.

In the Section on Muscle Biophysics, mechanisms of muscle regulation and contraction are studied, using both structural and functional techniques. A unique capability of the Laboratory is to bring to bear both structural (X-ray diffraction, electron microscopy) and functional techniques (force and displacement recording, radiation inactivation analysis) on specific questions in muscle physiology.

In the Section on Macromolecular Biophysics, lipid dispersions in water are investigated as models of the structure and physical states of lipids in cell membranes. Physicochemical relationships between diseased states in tissues and membrane bilayer instability is examined. Actions of ionizing radiation on macromolecules are used to determine the *in vivo* size of the functional units for different biological activities (hormone receptors, enzyme complexes, and regulatory units).

In the Section on Structural Biology, mechanisms of macromolecular assembly and structure-function relationships at the supramolecular level are investigated by high resolution microscopy in conjunction with digital image processing and by cryo-electron microscopy. Computer modelling is also used to integrate data from other biochemical and biophysical sources. Systems of interest include the structure and assembly of viruses, intermediate filaments including keratin filaments, clathrin and coated membranes, and other fibrous polymers and oligomeric protein complexes.

The Institute takes great pride in the scientific achievements of its intramural scientists and in their high reputation within the scientific community.

Lawrence E. Shulman, M.D., Ph.D.
Director
National Institute of Arthritis and
Musculoskeletal and Skin Diseases

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27000-27 LPB

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Mechanism of Muscular Contraction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard J. Podolsky	Chief	LPB/NIAMS
Others:	Leepo C. Yu	Research Physicist	LPB/NIAMS
	Robert Horowits	Senior Staff Fellow	LPB/NIAMS
	Maria Barbosa	Senior Staff Fellow	LPB/NIAMS
	Hidehiro Tanaka	Visiting Scientist	LPB/NIAMS
	Fu Zhao	Visiting Scientist	LPB/NIAMS
	Hiroyuki Iwamoto	Visiting Associate	LPB/NIAMS

COOPERATING UNITS (if any)

Dr. Alasdair Steven, Section on Structural Biology, LPB;Dr. Ellis Kempner, Section on Macromolecular Biophysics, LPB; Dr. Marinos Dalakas, NINCDS; Dr. Elizabeth Stephenson, N. J. Medical School; Dr. Koscak Maruyama, Chiba University, Japan.

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Muscle Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
6.5	4.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Striated muscle cells consist of three sets of filaments: (1) thick, myosin-containing filaments, which interdigitate with (2) thin, actin-containing filaments and (3) titin filaments, which center the thick filaments axially in the sarcomere. The titin filaments were found to be ideal elastic structures along their entire length in the I-band when they were extended either by (i) stretch of the relaxed sarcomere or (ii) calcium-activated movement of thick filaments. The thick filaments are arranged laterally on a hexagonal lattice, the stability of which is controlled by the presence of titin and/or nebulin. This was shown by degrading these very large proteins with high energy radiation and measuring the change in lateral order of the thick filament lattice following pH and temperature stress.

A widely accepted model of muscle contraction postulates that force is generated by "rocking" the head part of the myosin molecule after it attaches to the actin filament. This model is supported by X-ray reflections from fibers put into the rigor state and strained to various force levels which indicate that the long axis of the myosin head is more orthogonal to the actin filament when force is increased.

Single cell techniques were applied to skinned muscle fibers prepared from open muscle biopsies taken from patients suffering from Duchenne muscular dystrophy (DMD). Measurements were made of structural protein content, of calcium-activated tension (to test actomyosin function), and of resting tension (to assay the elasticity of the titin filaments). When sarcomere length was controlled and fibers were characterized as "fast" or "slow", DMD fibers were the same as control. Therefore, the defect in DMD leads to weakness by causing breakdown of fibers that are capable of developing normal force.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27001-15 LPB

RIOD COVERED

tober 1, 1988 through September 30, 1989

LE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Contractility of Skeletal and Smooth Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. Schoenberg, Medical Officer, LPB, NIAMS

Others: V. A. Barnett, Staff Fellow, LPB, NIAMS

COOPERATING UNITS *(if any)*

Laboratory of Molecular Cardiology, NHLBI (R. S. Adelstein, J. R. Sellers, M. A. Corson)

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Muscle Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

HECK APPROPRIATE BOX(ES)

(a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Despite the success of our 1985 model in quantitatively describing the behavior of weakly-binding M-ATP crossbridges, a serious impediment in totally understanding the interactions of myosin crossbridges with actin in fibers has been the failure of that model to quantitatively predict the fiber crossbridge detachment rate constants with nucleotide analogues bound at the active site. In FY '89 this difficulty was overcome with the demonstration that the reason the fiber detachment rate constants are much slower than predicted by the '85 model is because that model does not take into account that, in the fiber, crossbridges can bind with both of their subfragment-1 heads. During FY '89 it was shown that when the 1985 model is modified to explicitly take into account double-headed binding, the model quantitatively predicts the behavior of crossbridges under almost all conditions of interest.

In addition to the progress in understanding skeletal muscle crossbridge interactions, some progress was also made in understanding the regulation of smooth muscle. While the mechanism of activation by major smooth muscle agonists such as acetylcholine and histamine has been known for some time, very little work has been done with some of the lesser well-known agonists. Substance P, a neuropeptide agonist which may be important in the pathogenesis of asthma, seemed like an interesting candidate for study because, unlike many of the other agonists, it causes a rather slow and sustained contraction. Thus, in FY '89, we investigated the mechanism of Substance P activation. Surprisingly, its mechanism of action was, in regards, like that of acetylcholine: It was receptor mediated; it lead to phorylation of the 20 kDalton myosin light chain at the same serine residue phosphorylated by myosin light chain kinase *in vitro*; and finally, the rise in myosin light chain phosphorylation preceded the rise of tension. Notably, the smooth muscle's contraction velocity at light loads was, for a given level of light chain phosphorylation, significantly less with Subtance P than with acetylcholine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 27002-11 LPB

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Macromolecular Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Alasdair C. Steven, Visiting Scientist - Section Chief, LPB, NIAMS

Margaret E. Bisher, Microbiologist	LPB, NIAMS
Frank P. Booy, Visiting Scientist	LPB, NIAMS
Eva Kocsis, Visiting Fellow	LPB, NIAMS
Joseph R. Taylor, IRTA Fellow	LPB, NIAMS
Daniel J. Thomas, Visiting Scientist	LPB, NIAMS

COOPERATING UNITS *(if any)* Computer Systems Laboratory, Division of Computer Research and Technology, NIH (B.L.Trus); Biological Engineering and Instrumentation Branch, Division of Research Services, NIH (M. Unser); Dermatology Branch, NCI, NIH (P.M. Steinert); others as noted.

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Section on Structural Biology

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
05	05	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structures and mechanisms of assembly of biological macromolecules, macromolecular complexes, and subcellular structures, are studied, primarily by high resolution electron microscopy and computer image processing. Two projects completed over the past year concern (1) the nucleocapsid structure of a herpesvirus; and (2) mapping the biosynthetic pathways of filaggrin and loricrin, two major proteins of the outer layers of mammalian epidermis.

(1) Two DNA-free capsids may be purified from cells infected with equine herpes virus, type 1, of which "light" capsids are abortive, whereas "intermediate" capsids are related to a maturable precursor. We have performed a molecular inventory of these particles by combining scanning transmission electron microscopy with quantitative gel electrophoresis. The main findings are that their 150 hexons are hexamers of VP9(148kDa), and that "intermediates" contain an additional protein, VP22, that accounts for 10% of its mass. Three-dimensional reconstructions from cryo-electron micrographs reveal many details of the capsid structure, showing in particular, that all capsomers are traversed by radial channels, and that VP22 forms irregular 20-30nm aggregates inside the capsid shell. These aggregates may represent the remnants of morphogenetic cores in capsids interrupted in the process of DNA packaging.

(2) Immuno-cytochemistry with protein A-gold was applied to samples of newborn mouse skin. From the resulting labelling patterns, we infer that filaggrin precursor is sequestered in large irregular granules in stratum granulosum cells, from which it is released and processed to become integrated into the large bundles of keratin filaments that fill the cells of the stratum corneum. Loricrin initially accumulates in small round granules in stratum granulosum cells. Upon release from these granules, this protein becomes incorporated into the covalently cross-linked envelopes of stratum corneum cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27003- 30 LPB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biophysical Studies of Metabolic Activity and Control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ellis S. Kempner, Ph.D., Physicist and Chief,
Section on Macromolecular Biophysics LPB NIAMS

COOPERATING UNITS *(if any)*

Drs. R. Podolsky (NIAMS), P. Skolnick (NIDDK), E. Rabon (VA Los Angeles), D. Vessey (VA San Francisco)

LAB/BRANCH

Lab/Branch: Laboratory of Physical Biology

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda Maryland 20892

TOTAL MAN-YEARS: 2.0.	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) Target analysis of the inactivation of biological activity by ionizing radiation was applied to several major problems including the proton-potassium ATPase and the UDP-glucuronyltransferase enzymes.

2) Work was completed on a study of the mushroom tyrosinase enzyme which confirmed an assumption of target analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27004-20 LPB

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Dynamic Properties of Cell Membranes and Related Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Norman L. Gershfeld, Ph.D.	Research Chemist	LPB, NIAMS
	Lionel Ginsberg, Ph.D, M.D.	Visiting Scientist	

COOPERATING UNITS (if any)

Dr. Stanley I. Rapoport, LN, NIA
Dr. Jiri Safar, LCNSS, NINDS

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Section on Macromolecular Biophysics

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A general theory of lipid bilayer assembly has been proposed which asserts that the process is a critical phenomenon in which the growth temperature is a critical point. An important corollary of the theory states that when the critical conditions for bilayer assembly are violated normally stable membranes will spontaneously degenerate. Previous studies have demonstrated the relevance of this theory in various cellular systems. In the most recent studies the theory has been applied to bilayer assembly in normal and diseased neural tissue. The following systems have been examined:

a) Hamster synaptosomes and microsomes from animals that have been infected with Scrapie prions and normal control synaptosomes.
Total lipid extracts from normal synaptosomes yield critical bilayer assembly temperatures of 36±1°C as expected for this animal, while the corresponding lipid extracts from the infected tissue gave a value of 25°C.

b) Human brain tissue from Alzheimer patients. As reported previously total lipid extracts from tissues with lesions gave critical bilayer assembly temperatures 10-15 degrees below normal controls of 37°C. These studies were obtained with small amounts of proteolipid protein present. Present studies have developed methods for removing proteolipid protein from the lipid extracts without affecting lipid composition. Critical bilayer assembly temperatures are unaffected by the removal of this protein.

These results indicate for both the Scrapie infection and Alzheimer disease that a membrane lipid defect can be the basis for cellular degeneration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27005-07 LPB

IOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aggregation of Human Platelets Induced by Decompression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Makio Murayama Research Chemist LPB, NIAMS

COOPERATING UNITS *(if any)*

Dr. K.K Kumaroo, Biochemist, U.S. Naval Research Institute, Bethesda, MD

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS 1.0	PROFESSIONAL: 1.0	OTHER

CK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main thrust of the laboratory is focussed on the influence of pressure on the blood coagulation system, including both hemostasis and thrombosis. The molecular mechanism of platelet interactions in DIPA (decompression-inducible platelet aggregation) has been investigated for the past several years and is still continuing. We have found that the molecular mechanism of DIPA can be explained by the interactions of oppositely charged amino acid residues, i.e. positively charged arginyl residues interact with negatively charged aspartyl residues. We found this to be the molecular mechanism in AMS (acute mountain sickness). Our theory has been experimentally confirmed by using the frog. Vascular occlusion has been observed in the small blood vessels in the web of the frog's foot. We found that AMS can be prevented in the frog by the drug piracetam, administered by stomach tube prior to ascent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 27012-05 LPB

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural and Mechanical Properties of Muscle Fibers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Leepo C. Yu, Research Physicist, Laboratory of Physical Biology, NIAMS

Sengen Xu, Visiting Associate, Laboratory of Physical Biology, NIAMS

COOPERATING UNITS *(if any)*

Dr. Bernhard Brenner (University of Ulm, FRG)

LAB/BRANCH
Laboratory of Physical Biology

SECTION
Section on Muscle Biophysics

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 2.25	PROFESSIONAL: 2.0	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the outstanding questions in muscle research has been whether the crossbridges (myosin heads attached to actin) exhibit more than one attachment configurations during the ATPase cycle and whether the changes are associated with force generation.

It has been determined previously that crossbridges attached in the relaxed states at low ionic strengths are in the pre-force generation states. By obtaining X-ray diffraction data from skinned muscle fibers in relaxed, fully activated and rigor states at low ionic strength (50mM), we compared the configurations of crossbridges in states before, during and after force generation. The data suggests that distributions of mass in the muscle cells are distinct among these three states. The center of mass of the myosin heads appears to shift slightly away from the backbone of the myosin filament in transition from the relaxed to the active state and shifts further in transition to the rigor state. The finding gives the first indication that there are structural transformations in the attached crossbridges associated with force generation.

Crossbridges in a fully activated fiber are found to be rather stiff in the direction perpendicular to the axis of the fiber. The stiffness is of the same magnitude as that in the axial direction and exhibits the characteristics of a linear spring. Furthermore, the elasticity and the equilibrium position where the radial force reaches zero appear to be a function of the crossbridge state. The individual radial elasticity may turn out to be a sensitive indicator of the binding strength of the crossbridges to actin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AR 41020-22 ARB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of autoimmunity in mice with SLE-like illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I. Alfred D. Steinberg, Chief, Cellular Immunology Section, ARB, NIAMS
Daniel Kastner, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
William Gause, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Arthur M. Krieg, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Regina Kurrasch, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Tohru Takashi, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Dorothy Scott, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Mark Gourley, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Arthritis and Rheumatism

SECTION
Cellular Immunology

INSTITUTE AND LOCATION
NIAMS - Bldg. 10, Rm. 9N218, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	8	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our prior studies demonstrated substantial expression of 8.4 kb endogenous MCF related RNA in thymuses of autoimmune-prone but not other mouse strains. We have localized this defect to the mPT sub-set of MCF endogenous retroviral sequences. In an attempt to determine whether endogenous MCF sequences play a role in normal physiology, anti-sense oligonucleotides to various endogenous retroviral sequences were used to modulate spleen cell RNA synthesis. Anti-sense to MCF-env increased spleen cell RNA synthesis. The anti-sense did not stimulate cells from rats, which lack mouse endogenous MCF sequences, nor did it bind to non-retroviral bands on Northern analysis. We conclude that the antisense interfered with a normal inhibitory process in which retroviral sequences themselves, or via their protein product, inhibit lymphocyte activation.

Some effort has been devoted to basic studies of normal mice preparatory to investigations into abnormalities in murine lupus. Studies of normal thymocyte development have demonstrated that progenitor $CD4^-$, $CD8^-$ thymocytes were induced by IL 1 alone to express cell surface alpha,beta T cell receptor (TCR) by inducing expression of the limiting TCR alpha message.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41022-17 ARB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Therapeutic Studies in Murine Lupus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Alfred D. Steinberg, Chief, Cellular Immunology Section, ARB, NIAMS
Tohru Takashi, Guest Researcher, Cellular Immunology Section, ARB, NIAMS
Arthur M. Krieg, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
William C. Gause, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIAMS - Bldg. 10, Rm. 9N218, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Spontaneously autoimmune mouse strains provide good models for potential new therapies for humans with autoimmune diseases. Previous studies have suggested benefit of tumor necrosis factor alpha (TNF) given from early in life to lupus-prone mouse strains. We have performed additional studies of TNF in MRL-lpr/lpr mice. Such therapy prolonged survival when given to MRL-lpr/lpr mice from 6 weeks of age; when given to older MRL-lpr/lpr mice, it reduced lymphadenopathy but survival was not prolonged. These studies suggest that TNF may be useful as prophylaxis but not therapy.

Anti-IL 4 antibody given from birth to MRL-lpr/lpr mice retarded the development of lymphadenopathy and of splenic accumulation of CD 4$^-$, CD 8$^-$ T cells. These studies support a role for cytokines in the pathogenesis of murine lupus and point to the possibility that anti-cytokine antibodies may modify disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41023-15 ARB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Patients with Immune-mediated Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Alfred D. Steinberg, Chief, Cellular Immunology Section, ARB, NIAMS
Arthur Krieg, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Daniel Kastner, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIAMS - Bldg. 10, Rm. 9N218, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

mRNA hybridizing with probes specific for human endogenous retroviruses has been detected in leukocytes from patients with autoimmune diseases and normal individuals. Additional studies need to be performed with more specific subclass probes to determine whether or not there is disease specific expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41025-18 ARB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the cell surface receptor for IgE

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	H. Metzger	Chief, Sec. Chem. Immunol.	ARB/NIAMS
	S. Mao	Visiting Fellow	ARB/NIAMS
	S.C. Dreskin	Expert	ARB/NIAMS
	L. Miller	NRC Fellow	ARB/NIAMS
	G. Alber	Visiting Fellow	ARB/NIAMS
	B. Grasberger	Biologist	ARB/NIAMS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 5	PROFESSIONAL: 5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The receptor for immunoglobulin E (IgE) mediates the degranulation of mast cells and basophils and serves as an interesting example of the more general group of membrane-bound "Fc receptors". We previously studied the structure of the receptor using protein chemistry and then applied molecular genetic approaches. This led to the cloning of the genes for each of the subunits and their transient expression in COS cells as assessed by IgE binding. During the past year we have engaged in four principal types of studies and obtained the following major results: 1) investigation of the biosynthetic assembly of the receptor. Several precursors of the α chain were observed which appear to be incompletely glycosylated. The initial data suggest that the α and β are the first subunits to interact. 2) Reconstitution of intact receptors into liposome for functional studies. These studies show that receptors can be incorporated into liposomes, such that there are multiple, properly oriented receptors per liposome. No transmembrane signalling has yet been observed in such reconstituted systems. 3) Completion of studies on cell-free preparations that retain receptor-mediated transmembrane signalling. The latest results indicate that even after lysing (and depletion of >90% of intracellular soluble proteins) the cellular "ghosts" retain receptor-mediated hydrolysis of PI. However, further disruption to yield conventional membranes led to loss of all activity. These results suggest disruption of some important structural component. 4) initiated genetic studies to assess the role played by various parts of the receptor in assembly and surface expression, and in transmembrane signalling. Initial results have indicated: a) Receptors containing a variety of mutated subunits can be expressed on COS cells. However, on the latter neither they nor transfectedwild-type receptors signal such changes as an increase in {Ca2+} in or PIturnover. On the other hand, a receptor-less mouse mast cell line permanently transfected with the genes for the wild-type receptor shows good transmembrane signalling. We are currently attempting to make permanent lines transfected with mutant receptors.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AR 41040-17 ARB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of various cytotoxic drug programs in diffuse lupus nephritis.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Alfred D. Steinberg, Chief, Cellular Immunology Section, ARB, NIAMS
Mark Gourley, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Dorothy Scott, Medical Staff Fellow, Cellular Immunology Section, ARB, NIAMS
Susan C. Steinberg, Special Volunteer, Cellular Immunology Section, ARB, NIAMS

COOPERATING UNITS *(if any)*
James E. Balow, Senior Investigator, NIDDK
Howard A. Austin, Attending Nephrologist, Clinical Center
Foreign: NONE

LAB/BRANCH
Arthritis and Rheumatism

SECTION
Cellular Immunology

INSTITUTE AND LOCATION
NIAMS - Bldg. 10, Rm. 218, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A long-term follow-up was conducted this year of patients with diffuse lupus nephritis previously entered into therapeutic trials. Intravenous cyclophosphamide was found to be superior to oral cyclophosphamide and to prednisone alone in this long-term follow-up.

New patients are being randomized to receive (i) monthly IV-cyclophosphamide, (ii) monthly IV methylprednisolone, or (iii) both. For the portion of patients completing 1 year of study, clear-cut differences have yet to emerge [since it took > 5 years for differences to occur in prior studies, we believe that a longer follow-up is necessary for even trends to emerge].

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41048 10 ARB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Bacterial cell wall-induced arthritis and hepatic granuloma formation in the rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	R.L. Wilder	Sr. Investigator	ARB/NIAMS
	R. Lafyatis	Med. Staff Fellow	ARB/NIAMS
	J. Case	Med. Staff Fellow	ARB/NIAMS
	E. Remmers	Staff Fellow	ARB/NIAMS
	H. Sano	Visiting Fellow	ARB/NIAMS
	G. Kumkumian	Biologist	ARB/NIAMS
	E. Sternberg	Visiting Scientist	ARB/NIAMS, CNB/NIMH
	S. Aksentijevich	IRTA Fellow	ARB/NIAMS

COOPERATING UNITS (if any)
Laboratory of Chemoprevention, NCI
Clinical Neurosciences Branch, NIMH
Developmental Endocrinology Branch, NICHDD

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have established that streptococcal cell wall arthritis in LEW rats has many features in common with invasive tumors. For example, during the past year we have convincingly demonstrated that synovial tissues from arthritic rats express high levels of proteins like transin, MYC and SIS as is often observed in invasive tumors. We have more completely characterized the effects and sites of production of cytokines such as platelet derived growth factor, fibroblast growth factor, and transforming growth factor-beta. Specifically, local production of platelet derived growth factor, acidic fibroblast growth factor and transforming growth factor-beta by diseased synovium has been directly demonstrated. The persistent expression of acidic fibroblast growth factor is dependent on thymic-dependent immune mechanisms.

During the past year, we have extended our studies of neuroendocrine regulatory mechanisms. We have shown that the arthritis susceptibility of LEW rats has been shown to be secondary, in part, to a central nervous system defect in biosynthesis of corticotropin releasing hormone in response to cell walls and other stress mediators. Arthritis resistant F344 rats have a normal or hyperactive hypothalamic-pituitary-adrenal axis response. These observations provide new insights into potential pathogenetic mechanisms relevant to human disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41066 07 ARB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of synovial tissues from patients with RA and related conditions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	R.L. Wilder	Sr. Investigator	ARB/NIAMS
	R. Lafyatis	Med. Staff Fellow	ARB/NIAMS
	E. Remmers	Staff Fellow	ARB/NIAMS
	J. Case	Med. Staff Fellow	ARB/NIAMS
	G. Kumkumian	Biologist	ARB/NIAMS
	H. Sano	Visiting Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*

Laboratory of Chemoprevention, NCI

Laboratory of Molecular Biology, American Red Cross

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIH, NIAMS, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The single feature most characteristic of rheumatoid arthritis is tumorlike proliferation of the synovium. Transformed-appearing fibroblastlike cells and new blood vessels are the major proliferating cell populations. During the past year, we have obtained additional evidence that platelet-derived growth factor and transforming growth factor beta (TGF-beta) play major roles in regulating the growth and function of the fibroblast-like cells in diseased synovium. In addition, we have demonstrated the presence of acidic fibroblast growth factor, or heparin binding growth factor-1, in rheumatoid synovial tissues supporting the view that this cytokine is involved in driving neovascularization.

In related studies, we have provided evidence for synergistic and antagonistic interactions of platelet-derived growth factor and interleukin-1 on synovial fibroblast growth, prostaglandin E2 production and collagenase biosynthesis. Moreover, we have examined in detail, the transcriptional regulation of the collagenase gene by interleukin-1 and its inhibition by retinoids. We have provided evidence that collagenase transcription is regulated through a cis regulatory element, the TRE, and the transactivating factors, c-jun and cfos, which together comprise the AP-1 element.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41072-04 ARB

PERIOD COVERED October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cyclosporin A in Rheumatoid Arthritis (RA)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.H. Klippel, Senior Investigator, ARB/NIAMS
R.L. Wilder, Senior Investigator, ARB/NIAMS
D. Yocum, Guest Staff, ARB/NIAMS
L. Gerber, Chief, Rehab/CC
J. Minor, Pharmacy/CC
C. Yarboro, Nursing/CC
C. Berkebile, Nursing/CC
S.M. Wahl, CIS/NIDG
H.G. Preuss, GT. University

COOPERATING UNITS *(if any)*

NIDR, Rehabilitation/CC, Pharmacy/CC, Nursing/CC Foreign: None

LAB/BRANCH Arthritis and Rheumatism Branch

SECTION Connective Tissue Diseases

INSTITUTE AND LOCATION NIAMS/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated during the past year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41073-03 ARB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

High dose methotrexate with Leucovorin rescue in rheumatoid arthritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.H. Klippel, Senior Investigator, ARB/NIAMS
J.B. Shiroky, Former LOM/NIDR
B.A. Chabner, DCT/NCI
C.J. Allerga, DCT/NCI
G. Inghirmai, KDS/NIDDK

COOPERATING UNITS *(if any)*

DCT/NCI, KDS/NIDDK Foreign: None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS/NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated during the past year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO AR 41074 02 ARB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on etiology and pathogenesis of idiopathic inflammatory myopathy in humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P.H. Plotz	Sr. Investigator	ARB/NIAMS
	F.W. Miller	Sr. Staff Fellow	ARB/NIAMS
	R. Leff	Med. Staff Fellow	ARB/NIAMS
	D. Fraser	Med. Staff Fellow	ARB/NIAMS
	M. Joffe	Summer Program	ARB/NIAMS
	L. Love	NRSA Fellow	ARB/NIAMS
	K. Waite	Howard Hughes Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*

Nursing Unit   Clinical Center   NIH
Division of Rheumatology   Oklahoma Medical Research Foundation

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects   ☒ (b) Human tissues   ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Idiopathic inflammatory myopathy (poly/dermatomyositis) is an inflammatory disease of muscle in which characteristic autoantibodies occur. By applying a broad range of immunological, clinical, and epidemiological observations to a large (expanded in the past year to close to 200) group of patients, we have developed evidence for subsets of patients which strongly suggest that the disease can be divided into more meaningful groups with different etiology, pathogenesis and response to therapy. For example, patients with antibodies to the signal recognition particle have an abrupt, severe onset in the autumn; those with anti-Jo-1 have a less abrupt onset, most often in the winter and spring. Laboratory studies have shown that the major autoantibody, anti-Jo-1, which defines a clinically important subgroup of patients, appears to be an antigen-driven secondary response directed to a conformational, not a linear, epitope of histidyl-tRNA synthetase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO AR 41075 02 ARB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Picornavirus-induced chronic inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P.H. Plotz	Sr. Investigator	ARB/NIAMS
	F.W. Miller	Sr. Staff Fellow	ARB/NIAMS
	L. Love	NRSA	ARB/NIAMS
	R. Leff	Med. Staff Fellow	ARB/NIAMS
	D. Fraser	Med. Staff Fellow	ARB/NIAMS
	N. Marin	MARC Student	ARB/NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued to study the pathogenesis of EMC-221A-virus initiated inflammation in mice. In the past year we have extended observations to T-cell deficient nude mice but analysis of the data is not yet complete. We are currently assaying tissues from mice by the more sensitive PCR technique to extend earlier observations made by in situ hydridization. We have succeeded in making a full-length cDNA of the RNA genome and have expanded the bulk of the genome, including the troublesome 5 Poly-C tract, by PCR, en route to developing an infectious clone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41076-02 ARB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Therapeutic trials in idiopathic inflammatory myopathies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P.H. Plotz	Sr. Investigator	ARB/NIAMS
	D. Fraser	Med. Staff Fellow	ARB/NIAMS
	R. Leff	Med. Staff Fellow	ARB/NIAMS
	F.W. Miller	Sr. Staff Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*
Blood Bank Clinical Center, NIH
Unit of NM Diseases, NINCDS
Dept. of Rehabilitation Clinical Center, NIH

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Section on Connective Tissue Diseases

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Severe idiopathic inflammatory myopathies (polymyositis, dermatomyositis, and related diseases) that respond poorly to corticosteroids are difficult to treat. We have undertaken three therapeutic trials for the treatment of such patients: a) a controlled double-blind trial of plasmapheresis and leukapheresis; b) an open trial of monthly intravenous cyclophosphamide; c) a controlled, crossover trial of intravenous methotrexate with leucovorin rescue and a combination of methotrexate and azathioprine. The trial of apheresis continues; 35 patients have entered so far - 5 in the past year - with a goal of 40. The trial of i.v. methotrexate and combination therapy, begun in September 1987, has had 17 patients entered so far - 6 in the past year. We anticipate concluding the apheresis trial by the end of this calendar year.

The trial of cyclophosphamide was terminated after 11 patients had entered because of inefficacy and the results have been reported.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41077 02 ARB

PERIOD COVERED
October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Gene Expression in Normal and Impaired Fracture Healing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark E. Bolander, M.D.	Senior Staff Fellow	ARB, NIAMS
Others:	Seiya Jingushi,M.D.Ph.D	Visiting Fellow	ARB, NIAMS
	Michael Joyce, M.D.	IRTA	ARB, NIAMS
	Sean Scully, M.D.,Ph.D	IRTA	ARB, NIAMS
	Lance Macey, M.D.	Volunteer	ARB, NIAMS

COOPERATING UNITS *(if any)*
Laboratory of Developmental Biology and Anomalies, NIDR (Y. Yamada)
Department of Orthopaedic Surgery, Georgetown University (T. Martinelli)
Laboratory of Chemoprevention NCI (A. Roberts and M. Sporn)

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Orthopaedic Research Unit

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Md.

TOTAL MAN-YEARS: 3.5 man years
PROFESSIONAL: 3.5 professional
OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigations in the Orthopaedic Research Unit have focused on defining the relationship between the histological progression of fracture repair and the expression of genes for extracellular matrix proteins, the regulation of gene expression during repair by growth factors, and fracture repair in estrogen-depleted/osteoporotic fracture models. New projects include using a COL II transgenic mouse model to study collagen II gene expression during fracture repair, and investigations of the role of growth factors in the endochondral ossification front of the developing growth plate.

mRNA levels for extracellular matrix protein genes serve as markers for critical cellular events during fracture repair, yielding information on the regulation of gene expression and the initial events of protein synthesis.

Our studies implicate TGF-ß, aFGF, and PDGF as important regulators of cell proliferation, differentiation and protein synthesis during fracture healing. TGF-ß stimulates cellular phenotypic gene expression and proliferation in the fracture callus *in vitro*. TGF-ß stimulated mesenchymal cell proliferation and differentiation into both osteoblasts and chondrocytes with subsequent synthesis of cartilage and bone matrix respectively *in vivo*. aFGF stimulates chondrocyte proliferation and differentiation during fracture repair *in vivo*. PDGF initiated cell proliferation and extracellular matrix synthesis in both *in vitro* and *in vivo*. Surumin, a PDGF receptor antagonist, was used to block the effects of endogenous PDGF in both *in vitro* and *in vivo* fracture healing experiments.

Immunolocalization of FGFs and TGF-ß in the growth plate suggest that these growth factors regulate proliferation and early differentiation of chondrocytes. TGF-ß1 in the transverse septa may influence chondroclast-mediated resorption of the matrix and angiogenesis at the endochondral ossification front.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41078 02 ARB

PERIOD COVERED October 1, 1988 through September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Digital subtraction radiography of bone lesions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Mark E. Bolander, M.D. Senior Staff Fellow ARB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Orthopaedic Research Unit

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Md.

TOTAL MAN-YEARS: 0 man years
PROFESSIONAL:
OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Canceled

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41079 01 ARB

PERIOD COVERED

July, 1988 - July, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of the Central Nervous System in Susceptibility to Arthritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Esther M. Sternberg, Visiting Scientist, ARB, NIAMS; Guest Res., CNB, NIMH
Ronald Wilder, Senior Investigatior, ARB, NIAMS
Philip W. Gold, Chief; CNE, DIRP, NIMH
George P. Chrousos, Senior Investigator, DEB, NICHD
Aldo Calogeros, Guest Res., CNE, DIRP, NIMH
Harvey Whitfield, Medical Officer (Research), CNE, DIRP, NIMH
John Glowa, Research Psychologist, CNE, DIRP, NIMH
Mark Smith, Medical Officer (Research), CNE, DIRP, NIMH
W. Scott Young III, Senior Staff Fellow, LCB, NIMH
Sofia Aksentijevich, IRTA Fellow, NIAMS; Guest Res., CNB, NIMH
Piotr Zelazowski, Visting Fellow, NIAMS; Guest Res., CNB, NIMH
Leslie Crofford, Medical Staff Fellow, ARB, NIAMS
Craig Smith, Chemist, CNB, NIMH and Rachel Caspi, Senior Investigator, NEI

COOPERATING UNITS (if any)

COOPERATING UNITS: CNE; DIRP; NIMH; DEB; NICHD; LCB; NIMH; NEI

LAB/BRANCH

ARB, NIAMS/CNB, NIMH

SECTION

Inter-Institute Unit on NeuroEndocrine Immunology and Behavior

INSTITUTE AND LOCATION

NIAMS and NIMH

TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 3	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Summary of work:

A negative feedback loop exists between the immune and central nervous systems, in which immune/pro-inflammatory mediators signal the hypothalamic corticotropin releasing hormone (CRH) neuron to promote pituitary-adrenal activation and, hence, glucocortioicd mediated restraint of the immune response. We have found that increased susceptibility to streptococcal cell wall (SCW)-induced arthritis in the Lewis (LEW/N) rat is related to a defect in the central component of this negative feedback loop, resulting in deficient CRH responses to challenge with a variety of inflammatory mediators, including streptococcal cell wall peptidoglycan polysaccharide (SCW), interleukin-1 alpha (IL-1 alpha) , or the serotonin agonist quipazine. The relative arthritis resistance of F344/N rats compared to LEW/N rats is related to their intact HPA axis responses to these same inflammatory mediators. Physiologic replacement doses of corticosteroids (dexamethasone) in LEW/N rats significantly suppressed the severity of the arthritis in this arthritis susceptible strain, while interruption of the axis in F344/N rats with the corticosteroid receptor antagonist RU486 was associated with development of severe systemic inflammation and arthritis in response to SCW in this otherwise inflammatory disease resistant strain. Interruption of the F344/N HPA axis at the level of serotonin stimulation, with the serotonin antagonist LY53857, was also associated with development of arthritis. Thus, whether present on a genetic basis, as in LEW/N rats, or on a pharmacologic basis, as in RU486 treated F344/N rats, we have shown that interruptions of the HPA axis are associated with susceptibility to inflammatory disease. The deficient ACTH and corticosterone responses in LEW/N rats are secondary to a profound defect in regulation of CRH biosynthesis and secretion rather than to a defect in the CRH gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41080 01 ARB

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The use of MRI to detect inflammation im muscle of patients with myositis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	P.H. Plotz	Sr. Investigator	ARB/NIAMS
	D. Fraser	Med. Staff Fellow	ARB/NIAMS

COOPERATING UNITS *(if any)*

Department of Radiology, Clinical Center

NINCDS, NIH

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS,NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In an attempt to provide an improved assessment of muscle inflammation in patients with myositis, we have evaluated MRI. In a group of myositis patients, muscle biopsy, laboratory tests, and the T2-weighted image of the thighs were compared to an assessment of clinical disease activity. MRI was more sensitive in detecting inflammation than biopsy in these patients. The extent of muscle inflammatory changes could be assessed in patients since the entire muscle group is imaged.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AR 41081-01 ARB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of the Fc Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Jean-Pierre Kinet, M.D.	Visiting Scientist	ARB/NIAMS
	U. Blank	Visiting Fellow	ARB/NIAMS
	M.H. Jouvin	Visiting Associate	ARB/NIAMS
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	K. Huppi		Lab of Genetics/NCI
	H. Thompson		LCI/NCI
	D. Metcalfe		LCI/NCI

COOPERATING UNITS *(if any)*
Laboratory of Genetics, National Cancer Institute
LCI, National Cancer Institute

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The receptor with high affinity for IgE (FcϵRI) is a key molecule in triggering the allergic reaction. Therefore understanding its structure and its function is of considerable medical interest and may lead to new therapeutic approaches of allergic diseases. During the past year we have engaged in several types of studies: on the gene structure of FcϵRI, studies on the expression of the receptor by gene transfer, studies on the expression of the receptor during cell differentiation, studies on the receptor and IgE binding sites and studies on the structural relationship between FcϵRI and Fcγ receptors. These studies have yielded the following results. 1. With the exception of human beta, cDNA clones for alpha, beta and gamma of FcϵRI have now been isolated from three different species: rat, mouse and human. The genomic structure of the human gamma gene has been defined and the chromosomal localization of the subunits has been completed in mice and has begun in humans. 2. By using the techniques of gene transfer, non receptor-bearing cells have been induced to express receptors on their surface. The requirements for cell surface expression are different for the rodent and the human receptor. To understand this difference chimeric (rodent-human) alpha chains and other mutants of alpha have been produced. 3. Receptor expression comes at a very early stage of mouse mast cells' and human basophils' differentiation in IL3-dependent bone marrow cells' cultures. 4. To eventually allow production of crystals to study the receptor binding site, we have engineered truncated human alpha chains which are secreted by COS 7 cells. Production of a stable cell line is in progress. A 76 residues' peptide derived from the sequence of human IgE was shown to efficiently inhibit the binding of human IgE to human receptors. 5. Fc receptors from mouse macrophages and human NK cells are physically associated with gamma chains identical to those from Fc RI. These gamma chains have been cloned. Their presence is required for the expression of these FcγRs in transfected cells.

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